PYCNIDIAL PRODUCTION BY *DIAPORTHE VEXANS* (SACC. & SYD.) GRATZ AND THE PATHOLOGICAL HISTOLOGY OF INFECTED STEMS AND LEAVES OF EGGPLANT

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To my parents, brothers and their families, and sisters

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INTRODUCTION

Phomopsis disease of eggplant, commonly called Phomopsis blight and fruit rot, is an old and important disease found in all regions where eggplant is grown (8, 16). According to Chupp and Sherf (4), this disease ranks second only to bacterial wilt in destructiveness to the host in the tropics. In Florida, this has been considered the most serious disease of eggplant (8, 34, 35, 36). It has occurred in epiphytotic proportions in the state and has often resulted in severe losses (3, 34). The latest reported epiphytotic outbreak of the disease elsewhere occurred in Mauritius in 1964 (14).

A breeding program for control of the disease was initiated in Florida in 1942 (8) and 2 highly resistant hybrid varieties, 'Florida Market' and 'Florida Beauty,' were released in the winter of 1949-50 (9, 10). The varieties were still segregating for fruit color and type at the time of their release, as determined by subsequent test plantings (20, 21, 22). Nevertheless, the varieties became widely grown in Florida.

In spite of the recognition of Phomopsis blight as a serious disease, the numerous early accounts of its severity, and the breeding work done to find resistant varieties, very little is known concerning the effects of the causal organism on the cells and tissues of the host. The work reported herein was done to determine the histology of infection on eggplant and thereby gain insight into the mechanism of pathogenicity by Diaporthe vexans.

Since it was necessary to produce sufficient inoculum for this study, certain factors influencing the pycnidial formation of the fungus were also investigated. Throughout the investigation, the imperfect form of the fungus, Phomopsis vexans (Sacc. & Syd.) Harter was used. The name Phomopsis disease (29) is used in this paper.

This report is presented in 2 parts, one on some of the factors affecting pycnidial production, and the other on the pathological histology of the host.

SOME FACTORS AFFECTING THE PYCNIDIAL PRODUCTION OF *DIAPORTHE VEXANS* (SACC. & SYD.) GRATZ

Introduction

The physiology of *D. vexans* has not been studied extensively. Nolla (28) grew the fungus on 3 agar media and found that large numbers of pycnidia had formed by the 20th day of growth on oatmeal agar and corn flour agar. Few pycnidia were produced on dextrose nutrient agar; those produced were confined mostly to the edge of the colonies. Mycelial growth, however, was best in the dextrose nutrient medium. Palo (29) tried numerous agar media and found that steamed cornmeal, steamed rice, steamed eggplant stems and fruits, and steamed string beans were the most favorable for pycnidial production. Oatmeal, prune, Leonian's malt extract, and eggplant decoction agars were rated "fairly good," whereas pycnidia produced on cornmeal, cucumber seed, and string bean agars were "scant." Pawar and Patel (30) found "abundant pycnidial production on host decoction agar with and without dextrose, moderate in Richard's agar, poor in lima bean, oatmeal, plain, and Brown's agars, and nil on potato dextrose agar." Reports on the effect of temperature on pycnidial production were also few. Vasudeva (39) reported "optimum sporulation" at 20-25° C, whereas Pawar and Patel (30) found abundant pycnidial formation between 30-35° C, scanty between 11 and 28° C, and none at 30° C.

Since it is essential that each fungus isolate should be studied individually for sporulation (5), several tests were conducted. Here, no attempt was made to study the finer aspects of nutrition such as the carbon, nitrogen, vitamin, and other requirements of the fungus. The tests consisted merely of determining the agar media, temperature, and light most suitable to maximum pycnidial production. Unless otherwise specified, the plates were incubated at 28° C and pycnidia were counted 10 days after inoculation. Pycnidia from 12 representative samples from each plate were counted under a stereoscopic microscope. A sample consisted of a 4-mm disc of fungous growth cut with a No. 1 cork borer. The samples were taken from areas near the center, near the edge, and midway between the center and the edge of the plates. There were 4 replicates per treatment. In many instances, pycnidia that formed close to one another fused to form an aggregate. Because it was often difficult to determine how many pycnidia fused, an aggregate was counted as one. In experiments where artificial illumination was provided, a single 20-watt fluorescent lamp was used; this was placed about a foot above the plates.

Effect of Different Media on Pycnidial Production

Thirteen different agar media were tested to find out which favor abundant pycnidial formation. Media were: 1) Gerber's baby food agar (BFA), 2) Barley agar (BA), 3) Cornmeal agar (CMA), 4) Czapek's agar (CA), 5) Lima bean agar (LBA), 6) Metrecal agar (MA), 7) Oatmeal agar (OA), 8) Peptone-Phytone-Potato dextrose agar (PPP), 9) Potato dextrose agar (PDA),

10) Potato dextrose agar-Nutrient agar (PDA-NA), 11) Rice agar (RA), 12) V-8 juice agar (V8A), and 13) Wheat agar (WA). Oatmeal agar, PPP, V8A, CA, and MA were prepared as described elsewhere (26, 27, 32, 37, respectively). Oatmeal agar (OA) was prepared, using a modification of the technique described by Gooding and Lucas (15). Seventy g of oats were blended in 500 ml of water for about 5 min in a Waring blender. Fifteen g of agar were melted separately in 500 ml of water. The blended oats and melted agar were mixed thoroughly, placed in flasks, and autoclaved for 30 min or longer. In the case of CMA, LBA, PDA, and PDA-NA (15) (equal parts), Difco prepared media were used. Barley agar, RA, WA, (all at 7%), and BFA (6%) were prepared in the same manner as OA, except for RA, in which rice was pre-cooked prior to blending.

The different media were seeded with small bits of inoculum growing on OA and were kept in an incubator under constant illumination. The plates were observed periodically for growth and pycnidial production. Results are presented in Table 1.

The fungus fruited most heavily in CA but the pycnidia produced were very minute and mostly sterile; very few pycnidiospores were observed. The rest of the media supported abundant pycnidiospore formation. The fungus also differed in the manner of pycnidial production. Pycnidia were produced singly on BFA, CMA, LBA, MA, PDA, and V8A. On RA, about half of the pycnidia tended to aggregate. On BA, PPP, PDA-NA, OA, and WA, a majority of the pycnidia fused to form larger aggregates. The pycnidia formed also differed in size. Large pycnidia were produced on

Table 1. Pycnidial production of
Diaporthe vexans on different media

Agar media	No. of pycnidia per disc (4-mm dia.)[a]	Pycnidial Formation
Czapek (CA)	45	aggregate, very small
Rice (RA)	28	solitary-aggregate, medium-sized
Baby food (BFA)	25	solitary, small
Oatmeal (OA)	23	aggregate, large
Wheat (WA)	23	aggregate, medium-sized
V-8 juice (V8A)	18	solitary, small
Potato dextrose-Nutrient agar (PDA-NA)	17	aggregate, medium-sized
Barley (BA)	16	aggregate, large
Metrecal (MA)	15	solitary, small
Cornmeal (CMA)	14	solitary, small
Peptone-Phytone-PDA (PPP)	10	aggregate, medium-sized
Potato dextrose (PDA)	6	solitary, medium-sized
Lima bean (LBA)	6	solitary, small

[a] Average of 12 samples, replicated 4 times and taken 10 days after the start of the experiment.

BA and OA, small ones formed on BFA, CMA, LBA, MA, and V8A, and medium-sized pycnidia developed on the other media tested. Fig. 1 illustrates pycnidial production of the pathogen on some of the media used.

Oatmeal agar, RA, and WA were the most satisfactory media tested. Although pycnidial counts were high on BA and V8A, the pycnidia were smaller in size and occurred singly. The pycnidia produced on OA, RA, and WA were larger and two or more pycnidia often became aggregated to form a large fruiting body. Thus, it is evident that the actual number of pycnidia formed on these media is much more than those formed on BA or V8A.

In other tests involving pycnidial production, OA was selected over RA and WA because the pycnidia on OA medium were larger and because OA was easier to prepare than RA. Rice had to be cooked first before it could be blended to make RA.

Effect of Oatmeal Concentration on Pycnidial Production

D. vexans was grown on oatmeal agar containing 2, 4, 6, and 8% oatmeal. The cultures were incubated under continuous light. Results are presented in Table 2. Mycelial growth on OA containing 2% oatmeal was sparse and pycnidial counts were as high as in the 6 and 8% treatments. The pycnidia produced on 2% OA, however, were much smaller than those produced on 6 and 8% OA. Pycnidia produced were mostly solitary. Many pycnidia were produced on 4% OA; they were about as large as those produced on 2% OA. Pycnidial production on 6 and 8% OA

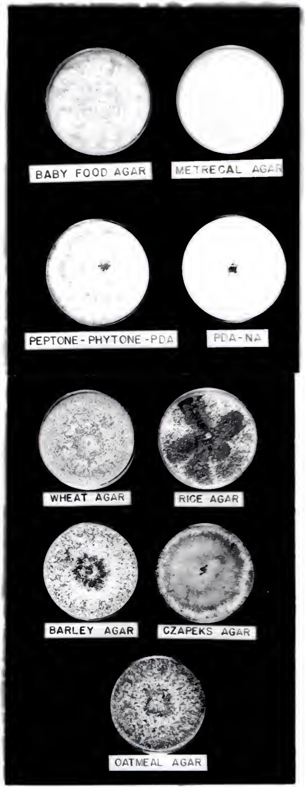

Fig. 1. Plate cultures of Diaporthe vexans after 10 days of growth on different media. Pycnidia appear as dark spots.

Table 2. Pycnidial production of *Diaporthe vexans* on oatmeal agar at different oatmeal concentrations

Percentage of oatmeal	No. of pycnidia per disc (4-mm dia.)[a]	Pycnidial formation
2	23	solitary, small
4	32	solitary-aggregate, small
6	26	aggregate, large
8	25	aggregate, large

[a] Average of 12 samples, replicated 4 times and taken 10 days after the start of the experiment.

was heavy, and pycnidia were larger than those formed on the 2 and 4% OA. A majority of the pycnidia coalesced to form large irregular fruiting bodies. Fungous colonies on 2, 4, and 6% oatmeal were smooth, but those on 8% oatmeal were rough. Fig. 2 shows the degree of pycnidial production at different concentrations of oatmeal.

This test showed that the fungus produced increasingly abundant mycelium, pycnidia, and larger fruiting bodies as the concentration of oatmeal was increased to 8%. Pycnidia were solitary at lower oatmeal concentration and aggregated at higher concentrations. Conceivably, a parallel situation would have resulted if other media, especially rice and wheat agars, had been used at different concentrations.

Effect of Light on Pycnidial Production

Preliminary observations showed that *D. vexans* produced very few pycnidia in an incubator without light, but produced many fruiting bodies in another incubator provided with artificial light. Tests were initiated, therefore, to determine the influence of light on the pycnidial production by this fungus.

One of the tests consisted of wrapping plates of freshly seeded OA (7% oatmeal) with aluminum foil to create a dark condition. Two small holes about 4 mm in diameter were punched in the foil to allow light to pass through. A set of plates completely covered with aluminum foil and another set without cover made up the controls. The plates were incubated at 30° C under a fluorescent light. On plates covered with foil in which holes were made, pycnidia were produced abundantly on

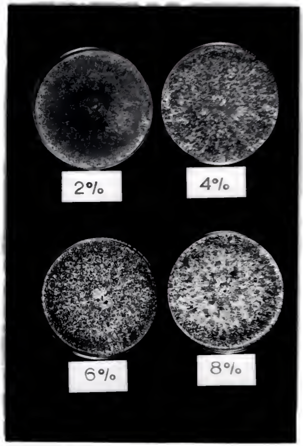

Fig. 2. Plate cultures of Diaporthe vexans grown for 10 days on oatmeal agar at different oatmeal concentrations.

areas exposed directly to light as well as on a small area immediately surrounding it (Fig. 3). Cultures completely exposed to light produced pycnidia abundantly but those completely covered formed no pycnidia. Furthermore, cultures exposed to light produced abundant fluffy aerial mycelium, whereas very little aerial mycelium was produced in the dark. Here the mycelium was thick and appressed to the medium. Similar results were obtained in later trials, when the fungus was grown on 6 and 7% oatmeal at 28° C.

In another experiment, the fungus was grown under 4 different sets of conditions on 6% OA. One set of plates was subjected to continuous light (about 200 ft-c), another set to continuous darkness, and a third set to a 24-hr alternate light and dark periods. A fourth set was placed in a small room near a lone window that faced north. This set received from 13 to 14 hr of natural daylight everyday. Room temperature during the day varied from 27.5-30.5° C during the experiment.

Results are summarized in Table 3. Cultures that were continuously illuminated were covered uniformly with black, mature pycnidia 7 days after inoculation. Growth was about the same on both sets receiving the 24-hr alternate light and dark periods and those receiving natural light. Here, although the mycelium reached the edge of the plates on the 7th day, pycnidial formation was slower compared to those under constant illumination. At this time, pycnidia were still forming in an area about 10 mm from the edge of the plates. On the 10th day, more pycnidia appeared in this area. The fluffy, aerial

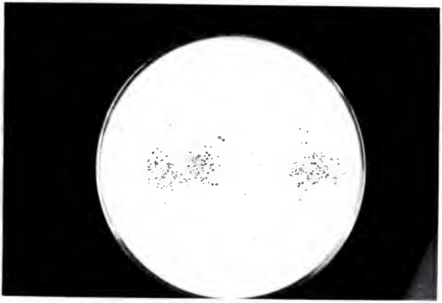

Fig. 3. A plate culture of Diaporthe vexans showing pycnidial formation on and around the vicinity of an area exposed to light. The plate was covered with aluminum foil with 2 small holes, each located between the center and edge of the plate.

Table 3. Pycnidial production of Diaporthe vexans under different conditions of illumination

Light treatment	No. of Pycnidia per disc (4-mm dia.)[a]
Continuous light (about 200 ft-c)	25
Alternate light and dark[b]	23
Natural room light[c]	21
Continuous darkness	1

[a] Average of 12 samples, replicated 4 times and taken 10 days after the start of the experiment.

[b] Twenty-four hr fluorescent light and 24 hr dark.

[c] Thirteen to 14 hr daylight and 10-11 hr dark.

mycelium was more dense than those produced under constant illumination. The plates kept continuously in the dark produced only a few scattered pycnidia on thick, white, felt-like mycelium (Fig. 4).

The relative age of the hyphae upon which light has a direct influence was determined. A set of plates was kept in the dark for 3 days after inoculation and the growing edge of the mycelium was marked with a glass marking pencil. The plates were then exposed to continuous illumination for 7 days. A second set of plates was subjected to 3 days of continuous light and was then kept in the dark for 7 days. A third set was subjected to 3 days of darkness, 1 day of light, and then 6 days of darkness. Controls were exposed to continuous darkness or continuous light for 10 days. Only visual observations were made in this experiment.

In the first and second set of plates, pycnidia were produced on mycelium that were formed in the dark. Pycnidial production was much heavier, however, on mycelia that were continuously exposed to light. In the second set of plates, for example, heavy pycnidial formation was confined to the central, circular area starting from about 1/2 cm behind the edge of the mycelial growth at the time of placement in the dark. In the third set of plates, heavy pycnidial production occurred in an area near the edge of the mycelium that was exposed to light. This area formed a conspicuous dark ring on the plate. Heavy pycnidial production occurred uniformly in cultures exposed continuously to light, but only a few scattered pycnidia formed in cultures kept in darkness.

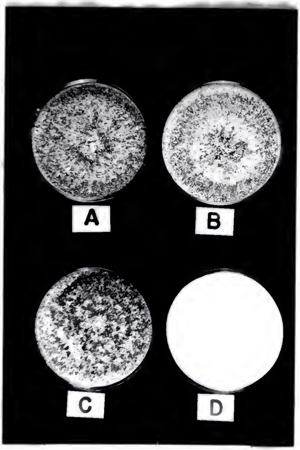

Fig. 4. Plate cultures of Diaporthe vexans grown for 10 days under different conditions of illumination. A) Continuous light, and B) 24-hr alternate light and dark inside an incubator; C) natural light under ordinary room conditions; D) continuous dark inside an incubator.

The results of tests described above show that light is required for abundant pycnidial formation. The 1-day alternation of light and dark did not decrease the number of pycnidia but certainly delayed their formation. This delay was more apparent during the first 7 days of incubation. Light also exerted its stimulatory effect near the growing tips of the hyphae. This confirms previous observations that very few pycnidia were produced when the fungus was subjected to continuous light after it had grown in continuous darkness for a week or longer. This observation is also similar to a report by Brown (2) on the sporulation of certain strains of *Fusarium*. He concluded that the most sensitive region of the colony to light is a short distance behind the growing tip. The presence of some pycnidia on areas previously grown in the dark and then exposed to light may be due to a few hyphal filaments formed on that area during the subsequent exposure to continuous illumination. Those formed in the dark on extension of mycelia previously exposed to light may be due to the ability of a few hyphae to transmit the pycnidial-forming stimulus of light to their filaments that are subsequently formed. Those formed on cultures placed continuously in the dark may be due to a very few hyphal elements that already possess this capacity to form pycnidia.

Effect of Temperature on Pycnidial Production

This test was made to determine satisfactory temperatures for pycnidial formation of this particular isolate of *D. vexans* and to compare its behavior with other isolates (30, 39). The fungus was grown on OA (6% oatmeal) at 20, 25, 30, and 35° C, under continuous light (about 200 ft-c).

On a per plate basis, more pycnidia formed at 30 than at 20 or 25° C. The rate of mycelial growth at 25 and 30° C was approximately the same; the mycelium covered the plates in 7 days. Mycelial growth and pycnidial production at 20° C was slower. The fungus reached the edge of the plates in 10 days, but at that time many of the pycnidia in that region were still immature. Fluffy, aerial mycelium covered the plates. At 20° C, many mature pycnidia near the center of the plates had oozed out spores 7 days after the plates had been inoculated; only very few pycnidia had released spores at the other temperatures. The fungus grew as well at 35 as at 25 and 30° C for the first 3 or 4 days. After that, however, growth rates were greatly reduced in cultures kept at 35° C. At the end of the test, only about 1/3 of the plate area was covered with fungous growth. Pycnidial production was heavy on areas containing mycelial growth, but pycnidia were smaller than those produced in cultures incubated at the 3 lower temperatures. On the basis of areas containing mycelium, pycnidial production at 35° C was 21, 36, and 40% more than that at 30, 25, and 20° C, respectively. On a per plate basis, however, the reverse was true; the number of pycnidia at 30, 25, and 20° C were 54, 43, and 40% more, respectively, than at 35° C. Results of these tests are summarized in Table 4 and illustrated in Fig. 5.

The present isolate of D. vexans produced the most number of pycnidia at 30° C on a per plate basis and covered

Table 4. Pycnidial production of Diaporthe vexans at 4 different temperatures

Temperature (°C)	No. of pycnidia per disc (4-mm dia.)[a]
20	20
25	21
30	26
35	33[b]

[a] Average of 12 samples, replicated 4 times and taken 10 days after the start of the experiment.

[b] Average of 4 samples, replicated 4 times. Counts were based only on areas with mycelial growth.

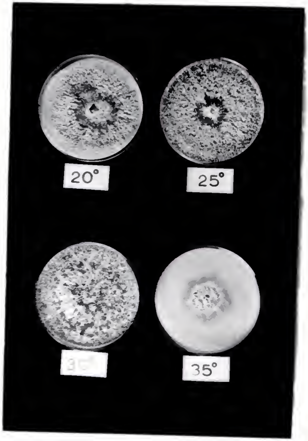

Fig. 5. Plate cultures of Diaporthe vexans grown for 10 days at 4 different temperatures (°C).

the plates earliest with pycnidia at this temperature. Growth was definitely inhibited at 35° C, but pycnidial formation was heavy in areas with mycelial growth. The present observations differ from the report of Vasudeva (39) that "optimum sporulation" was at 20-25° C, assuming that he meant pycnidial formation for "sporulation." In this test, no temperature lower than 20° C was tried, but a trend towards slower growth was observed from 25-20° C. Conceivably, a slower growth and formation of fewer pycnidia can be expected at temperatures lower than 20° C. The present test also showed maximum pycnidial formation at 30° C, on a per plate basis but probably more pycnidia can be produced from 30-34° C on the same basis. It appears that 30-35° C is the optimum for pycnidial production for this isolate. The present observations slightly differ from the report of Pawar and Patel (30), who demonstrated that few pycnidia were produced between 11 and 28° C. Results reported here, however, fully agree with their other statement (30), that many pycnidia were formed between 30-35° C.

Discussion

Some factors that favor abundant pycnidial production of *D. vexans* (Sacc. & Syd.) Gratz were revealed in this study. Among the several media tested, oatmeal, rice, and wheat agars were the most satisfactory. The test on the different concentrations of oatmeal showed that the fungus produced more and larger pycnidia at increasing oatmeal concentrations. Six per cent oatmeal, however, seems to be the limit, since there was not much difference in number and size of pycnidia between

6 and 8% oatmeal. It is also undesirable to increase further the oatmeal concentration beyond 8%, because the resulting medium becomes thicker and harder to transfer to plates.

The organism was also able to produce pycnidia abundantly from 20-30° C on a per plate basis. It is conceivable that this range can be increased by at least 2 or 3 degrees both beyond the lower and upper limits.

Light was indispensable to abundant pycnidial production. The difference in pycnidial production was very striking between treatments that received light and those that did not. Furthermore, light exerted its stimulatory effect a short distance below the hyphal tips. With these findings, the pathogen can now be added to the list of fungi that require light for abundant sporulation. Among these are Diaporthe batatatis Harter & Field (18), D. phaseolorum (Cke. & Ell.) Sacc. (19), and D. sojae Lehman (25).

It would be interesting to know the effects of light quality, intensity, and duration, the effects of a wider range of temperatures, the finer aspects of nutrition, and other factors on the production of pycnidia. These, however, are beyond the scope of the present study. The objectives of the present study have been fully realized; there is now sufficient information to permit ready production of an abundance of inoculum in the laboratory. Large numbers of viable spores are produced within 7-10 days in illuminated cultures in an OA medium containing 6% oatmeal.

PATHOLOGICAL HISTOLOGY OF DIAPORTHE-INFECTED EGGPLANT

Introduction

Early reports indicate that D. vexans does not require injury to penetrate the host (12, 28, 29). Nolla (28) implied that the germinating spore may enter the suscept through the stomata, wounds, or the unbroken cuticle. By clearing and staining inoculated leaves, Howard and Desrosiers (23) demonstrated that the pathogen entered on both sides of the leaves by direct penetration. They found no germ tube entering open stomata, even though they observed many spores germinating nearby. They also suggested that the germ tube apparently penetrated the epidermal cell by dissolving a part of the wall and then progressed through the tissues intracellularly (23).

Nolla (28) observed that hyphae became much branched in an epidermal cell in which they entered; from there they invaded the surrounding epidermal and cortical cells by piercing the walls. Invaded epidermal and cortical cells soon become hydrotic; browning occurred in the epidermal cells and 2 or 3 layers of cortical parenchyma (28). The brown discoloration did not reach the vascular elements, but became visible on the veins of leaves and fruits (28).

This study was conducted to determine how the fungus affects the cells of the host and the response of the host to the invading organism. Since very little is known concerning the mode of ingress and spread inside the host, this aspect of pathogenesis was also reinvestigated.

Mode of Ingress

Seeds of eggplant, Solanum melongena L. 'Florida Market,' were obtained from commercial seed stores and planted in the greenhouse in 4-in and 6-in pots. Leaves from mature plants were excised and placed inside large petri dishes lined with moist filter paper. The leaves were divided into 2 lots; one was inoculated on the upper surface and the other lot on the lower surface. Inoculum was prepared by growing the fungus in 6 or 7% oatmeal agar at 28-30° C under continuous light (about 200 ft-c). Illumination was provided by placing a 20-watt fluorescent bulb about a foot above the plates. Spore suspension was prepared from 7- to 10-day-old cultures. One eighth of a plate culture was placed in 200 ml of water and shaken gently for about 5 min until a good suspension of spores was obtained. The resulting spore suspension was transferred to another container and used as inoculum.

Inoculation was done by placing drops of pycnidiospore suspension on the leaf surfaces. The inoculated leaves were incubated at 28° C. Leaf disc samples were taken 6, 12, 24, 36, 48, 60, and 72 hr after inoculation. This was done by punching the area below the drop of inoculum with a cork borer. The samples were decolorized by 2 methods. Some were kept for 1 day in FAA, 1 day in 50% lactic acid, and were then transferred to 95% ethyl alcohol (7). Others were placed directly in 95%

ethyl alcohol and were kept there until all color had disappeared. The decolorized leaf discs were stained in lactophenol blue, washed and mounted in clear lactophenol, and examined.

No spores germinated in 6 hr, but about 80% germinated in 12 hr. No penetration was observed then. After 24 hr, germ tubes of about 30% of the germinated spores had penetrated both sides of the leaf. Penetration was usually direct (Fig. 6), but frequently occurred through the stomata (Fig. 7-A). In direct penetration, most of the germ tubes penetrated either between 2 adjacent cell walls or on the outer walls of cells themselves (Fig. 6). Germ tubes penetrated between a guard cell and the adjacent epidermal cell and also through closed stomata. Most of the germ tubes penetrating at this time were short but large; many produced a swelling at the point of entry and a faint outline of an infection peg could be seen (Fig. 6-B). The point of entry of the germ tube was surrounded by a yellowish to brownish discoloration (Fig. 6-A).

More spore germination and penetration occurred after 24 hr. At 60 hr, some cells that were penetrated turned yellowish to brownish. It was also at this time that water-soaked areas became evident on the leaf in the area of inoculation. At 72 hr, the yellow to brown discoloration in many cells had become more intense.

Penetration was mostly direct when midribs, veins, petioles, and stems were inoculated. A stem section from a damped-off seedling also revealed that the fungus can enter young stems through the stoma (Fig. 7-B).

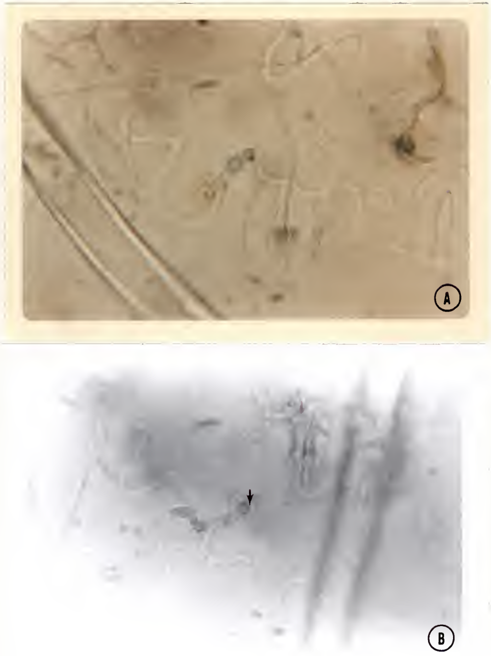

Fig. 6. Ingress by direct penetration. A) Germ tube penetrating an epidermal cell 72 hr after inoculation. Note the discolored area around the appressorium (1250X). B) Germinating spore showing a faint outline of an infection peg (arrow) coming out of the appressorium (1250X).

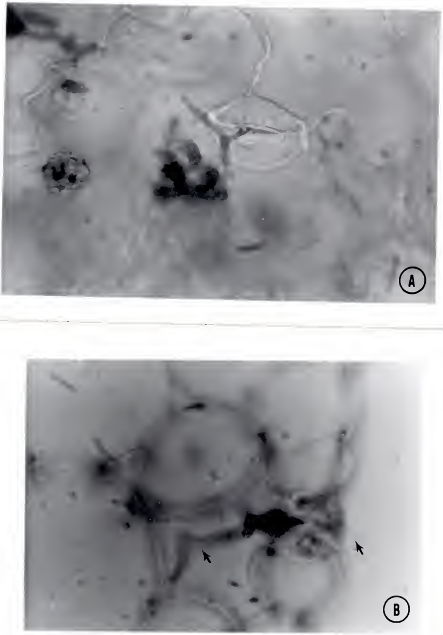

Fig. 7. Ingress through the stomata. A) A hyphal branch entering a leaf stoma (1250X). B) Cross section of a stem of a damped-off seedling showing a hypha that has entered a stoma. A portion of the same hypha at a different depth of focus is shown with arrows. The section is 50 u thick (1250X).

The mode of ingress of the pathogen was also tested on leaves of another plant, tentatively identified as Solanum aff. melogena (horticultural variety), using the same procedure used on the test plant 'Florida Market.' Spore germination, germ tube morphology, methods of penetration and presence of discolored halos around the points of entry were similar to those observed on the test plants of the Florida Market variety.

The present observations differ slightly from the report by Howard and Desrosiers (23), who observed only direct penetration but no stomatal entry. This report, however, confirms the suggestion by Nolla (28) that germ tubes may enter through stomata. Although the fungus can penetrate in 12 hr, the discoloration of the whole cell may not take place until at least 60 hr after inoculation. This coincides with the appearance of the first visible symptoms of the disease.

Mode of Internal Spread

Plants about 3-4 in. high or in their 4-6 leaf stage were inoculated with a spore suspension of the pathogen. The inoculum was prepared as described in the previous experiment of the mode of ingress. The inoculum was sprayed on plants by means of a de Vilbiss atomizer until the point of run-off. The inoculated plants were watered thoroughly and were incubated in a plastic box for from 4-7 days.

Sections of lesions on leaves and stems were cut by the free-hand, freezing microtome, or rotary microtome methods. Sections in the freezing microtome were cut from 25-40 u thick.

Tissues to be cut in paraffin were fixed in formalin-acetic acid-alcohol, dehydrated in the tertiary butyl alcohol series (24) and embedded in Paraplast. Sections were cut 10 u thick on a rotary microtome and were stained with either safranin-fast green or thionin-orange G (17).

When the fungus invaded an epidermal cell, that cell ultimately turned brown and collapsed. The fungus spread intra- and intercellularly. Hyphae were observed inside of and between epidermal, palisade, and mesophyll cells of infected leaves. Hyphae colonized these cells and formed pycnidia subepidermally. Pycnidia formed on the laminae, veins, or stems, and eventually became erumpent (Fig. 8). The fungus spread similarly in diseased petioles and stems. This eventually caused the early death of both leaves and stems. This premature death of leaves, however, also occurred in case of heavy infection of the lamina alone, or coupled with petiole infection up to the cortex.

The fungus killed the cells only after it had penetrated them. The wall of a penetrated cell turned brown at the point of contact with the hypha. Later the whole cell turned brown and the contents became coarsely granular (Fig. 9). The cell walls lost their integrity, the cell collapsed, and the contents apparently dissolved. Infected cells stained red in safranin and fast green whereas the healthy cells were green. The brown walls seen in fresh sections were also stained red in paraffin sections. Also evident in the paraffin sections

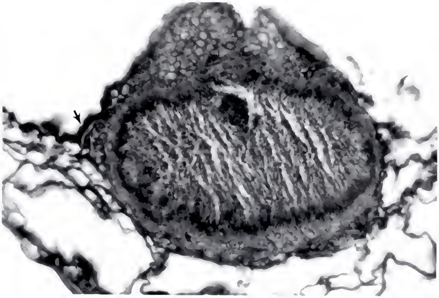

Fig. 8. Section of an erumpent pycnidium of Diaporthe vexans from an infected main vein of a leaf showing (arrow) portion of the dead epidermis (500X).

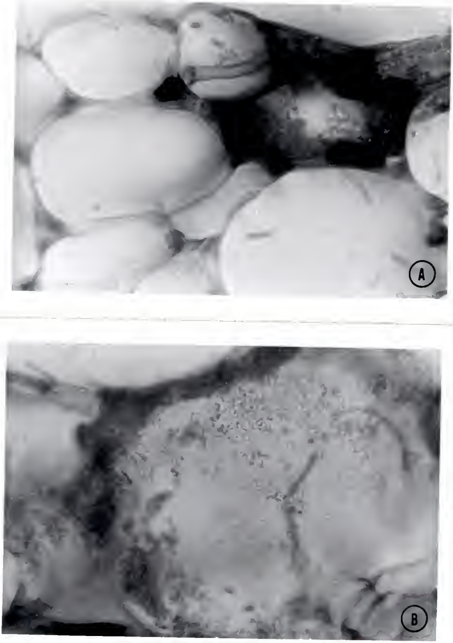

Fig. 9. Intracellular hyphae of *Diaporthe vexans* in diseased eggplant. A) Hypha inside an epidermal cell of a petiole penetrating a neighboring cell. Cells on the right are already destroyed as shown by the darkening of their walls and the transformation of their contents into granular materials. Note that the walls of the cell occupied by the fungus have not yet turned completely dark (1250X). B) Hypha inside a cortical cell whose contents have become coarsely granular and whose walls have turned brown and partially dissolved (1250X).

were the walls of collapsed cells. The walls looked flattened and appeared to separate into individual thread-like particles.

No hyphae were observed inside phloem or xylem vessels, but these cells showed the same changes as invaded cells of the cortex and epidermis. Some sieve and tracheary elements became brown, collapsed, and their wall components separated (Fig. 10). When sections having affected vessels were stained with phloroglucinol, the collapsed vessels and those visibly affected were brown in color; apparently unaffected vessels adjacent to these cells, however, were reddish-purple. Other vessels far from the infected area were also reddish-purple. Although no hyphae were observed inside affected vessels, the possibility of invasion is not discounted.

The present observations on the mode of spread of the pathogen inside the host and the discoloration of the host cells agree with Nolla's (28) report, except that he reported that browning occurred only on the epidermis and the 2 or 3 layers of cortical parenchyma. Although he observed browning on veins of leaves and fruits, he observed no browning of the vascular elements of stems and petioles.

Tests for Pectolytic and Cellulolytic Enzymes

The difference in staining reaction between healthy and diseased cells to safranin-fast green stain, and the browning, colapsing, and the seeming separation of walls of diseased cells into individual thread-like particles suggested enzymatic breakdown of walls of diseased cells. To determine whether the fungus produce pectolytic and cellulolytic enzymes, the following tests were conducted.

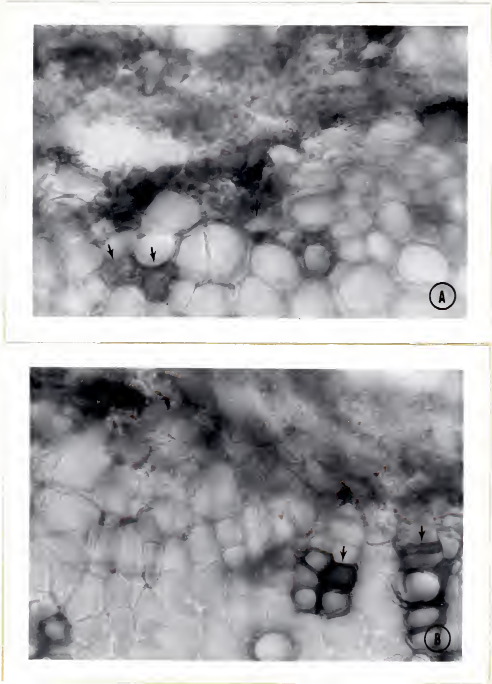

Fig. 10. Cross sections of a petiole of eggplant infected by Diaporthe vexans and stained with phloroglucinol, showing xylem vessels in various stages of destruction. A) Section showing 2 destroyed, brown vessels on the left and one near the center (arrows) (500X). B) Section showing brown vessels (arrows); the rest are reddish-purple (500X).

Staining for Pectin Pectin was stained by means of the hydroxylamine-ferric chloride reaction (31). Free-hand cross sections of petioles and stems were usually studied. Healthy tissues showed that the thick-walled collenchyma cells, which are rich in pectic substances (13), were stained distinctly red. The inner wall of epidermal cells, the chlorenchyma, the rest of the cortical cells, and the pith were pink, the xylem vessels were colorless, whereas the rest of the vascular elements were red (Fig. 11-A). Walls of dead cells, which had not fully disintegrated, were not stained. Some of the infected cells appeared pinkish, but were always fainter in color than the corresponding healthy cells. The area between healthy and attacked cells was usually dark (Fig. 11-B, 12-A). Those cells below the advancing pathogen that responded by cell division retained their color for a while, but these became colorless as they became infected (Fig. 12-B). This was specially evident in collenchyma cells; as they elongated and later divided, the walls became thinner, and correspondingly the color became lighter.

This histochemical test showed that the fungus degraded pectic components of the host cell walls. It was observed, however, that the degradation occurred slowly; even in plesionecrotic cells, the pinkish color was still evident. The complete disappearance of the pink color occurred only in holonecrotic cells. It is probable that the pectolytic enzymes produced by the pathogen probably were in low concentration or were partially inactivated by other compounds present in the vicinity of the affected areas.

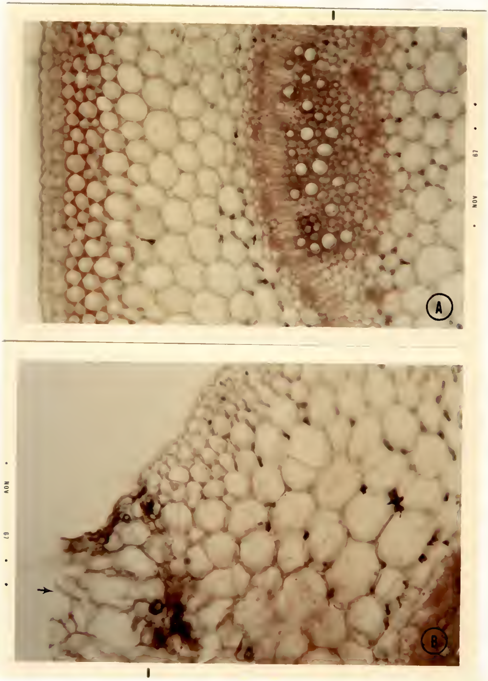

Fig. 11. Cross sections of healthy and infected petioles of eggplant stained for pectin. A) Section of healthy tissues. Note the distinct red color of the collenchyma tissue (125X). B) Section of an infected tissue showing portion of infected (arrow) and healthy cells. Note the absence of red color on the diseased cells (125X).

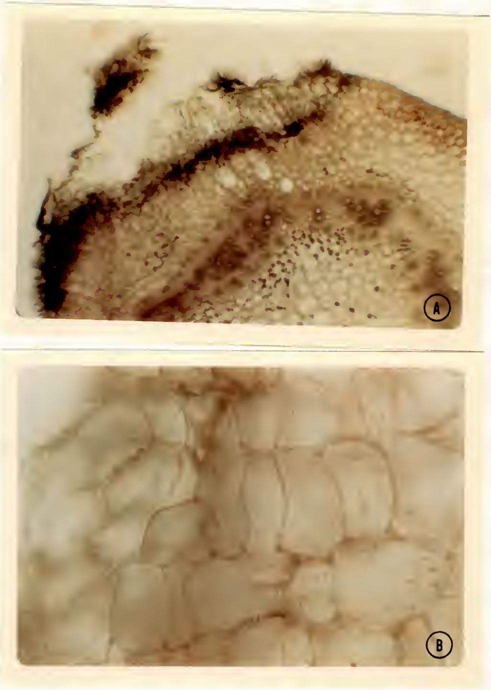

Fig. 12. Cross sections of eggplant stems infected by Diaporthe vexans and stained for pectin. A) Section showing the absence of red color on the infected area and the red color of healthy tissues (50X). B) Closer view of a section showing the difference in the coloration between healthy and diseased cells (500X).

Staining and Birefringence Tests for Cellulose Sections of stems and petioles were cut with a freezing microtome and were stained for cellulose by means of the zinc-chlor-iodide reaction and the IKI-H_2SO_4 method (24).

According to the zinc-chlor-iodide test, cell walls containing large amounts of lignin, cutin, suberin, or chitin will stain yellow to orange whereas walls containing large amounts of cellulose will stain blue, provided lignin, cutin, suberin, or chitin are removed. Sections of young, healthy petioles and stems subjected to this test showed that the epidermis, cortex, and phloem were faint blue to purplish, whereas the xylem vessels were purplish when young and yellow when older. Older, healthy petioles and stems showed the same color reaction except for the outer wall of the epidermis, which appeared yellowish. Sections with lesions showed that walls of infected and dead cells of the epidermis and cortex did not stain blue but were yellow (Fig. 13). This staining reaction was consistent with lesions from both young and older petioles and stems.

In the IKI-H_2SO_4 method, cell walls that contain cellulose will appear blue and those with lignin will appear orange to yellow. In the present test, sections of healthy petioles and stems showed that in the epidermis, the outer wall stained orange-yellow and the rest of the wall blue. The cortex was blue, the xylem vessels dark red, and the phloem dark blue. Diseased epidermal and cortical cells stained orange to red. When more acid was added to the mounts, the outer wall of the epidermis, the trichomes, the diseased cells, and the xylem vessels turned from red to orange, then yellow, and ultimately became colorless. On the

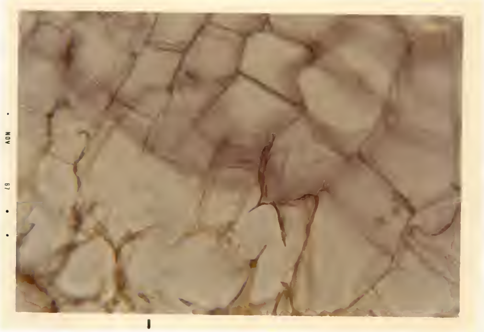

Fig. 13. Cross section of an eggplant stem infected by *Diaporthe vexans* showing the diseased (yellowish) and healthy (purplish) portions of the cortex. The section was stained for cellulose using the zinc-chlor-iodide test (500X).

other hand, the other tissue constituents turned darker in color and eventually disintegrated. It had also been observed that while acid was disintegrating dead cells adjacent to newly infected cells, certain portions of them showed a blue color, together with the orange and red colors.

Cellulose is crystalline in nature and as such it is expected that cell walls will be birefringent when viewed under polarized light (13). To find out if changes had occurred in the cellulose component of walls of diseased cells, sections of infected stems containing both diseased and healthy cells were examined under polarized light. Healthy cells showed a strong birefringence of their walls (Fig. 14-A). Walls of infected cells adjacent to healthy cells which showed early infection appeared darker. In cells that had collapsed and were brownish in color, the walls appeared much darker, although the birefringence was not totally lost (Fig. 14-B). Another check was made by examining sections from a stem that have been previously injured with a hot glass rod. Although the cells appeared collapsed, no difference in the birefringence of the walls was noted between the injured and healthy cells.

Results reported above suggest that cellulolytic enzymes were also involved in the degradation of the host cell walls. As in pectin degradation, the cellulose breakdown also appeared slow. In the IKI-H_2SO_4 test for example, the blue color appeared on collapsed dead cells that were previously stained red after the addition of acid caused swelling of the walls. Admittedly, this staining procedure provides only a rough estimate of the

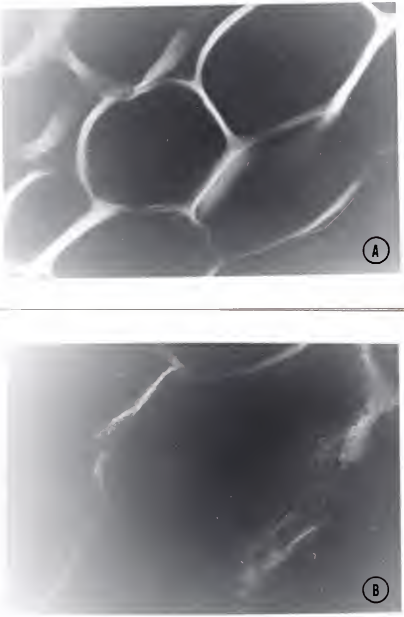

Fig. 14. Cross sections of eggplant stems under polarized light. A) Healthy cells showing strong birefringence of the cell walls (400X). B) Diseased cells showing a reduction in the birefringence of the cell walls (400X).

localization of cellulose, since hemicellulose may interfere in the staining reaction (24). The observations in the birefringence of cell walls, however, confirmed the staining tests. Dead cells whose walls were disintegrating still showed a slight birefringence, an indication that perhaps cellulose was only being slowly degraded. As with the pectolytic enzymes, this slow action may be due to a low concentration of the cellulolytic enzymes produced by the fungus or to a partial inactivation of these enzymes by other substances.

The Histologic Reaction of Eggplant to Diaporthe Vexans

One of the objectives of the present work was to learn the histologic response of eggplant to infection by *Diaporthe vexans*. Since many of the stem and petiole lesions were raised and limited in size, it was suspected that a certain type of defensive mechanism was being exhibited by the host. Thus, histological and histochemical tests were made to learn the host reaction during pathogenesis.

Histology of Damped-off Seedlings Infected seedling stems were cut with the freezing microtome at the stem lesions near the soil line. All cells of the epidermis, cortex, and outer phloem were collapsed and some cells were more intensely brown than others. Although collapsed, most were not entirely degraded and many chloroplasts were still intact. Hyphae were throughout the diseased stems. Xylem vessels had turned brown; some had collapsed, but the majority still retained their form.

Sections from brownish, elongated lesions of infected seedlings were also examined. In some sections the entire cortex was infected. A few cells below the infected region had divided, but most of them had not. Most of the healthy cells below the diseased area, however, enlarged and had thinner walls. In other sections cut from sunken lesions near the tip of a seedling stem, many cells below the infected cells had become elongated and enlarged; there was little cell division (Fig. 15-A).

The above observations how that the host is very susceptible when immature. The cells were readily killed by the pathogen, and there was no change in size and number of neighboring cells. As tissues become mature, however, the host began to show signs of a hypertropic response through enlargement of cells below the infected region.

Histology of Petioles and Stems With Lesions Lesions on stems and petioles first started as watersoaked, slightly sunken areas. These sunken areas enlarged faster longitudinally than laterally and resulted in elongated or lens-shaped lesions. The lesions became brown to dark brown or black in color. On immature tissues, lesions usually became sunken and remained this way. On mature tissues, however, the dark lesions became raised around their borders and eventually the whole lesions became elevated. The lesions enlarged very slowly when these raised areas were formed, and sometimes the growth of the lesions was stopped. As the tissues became older, some of the dark brown dead areas sloughed off and left a rough light brown surface.

Sections of sunken lesions of immature petioles and

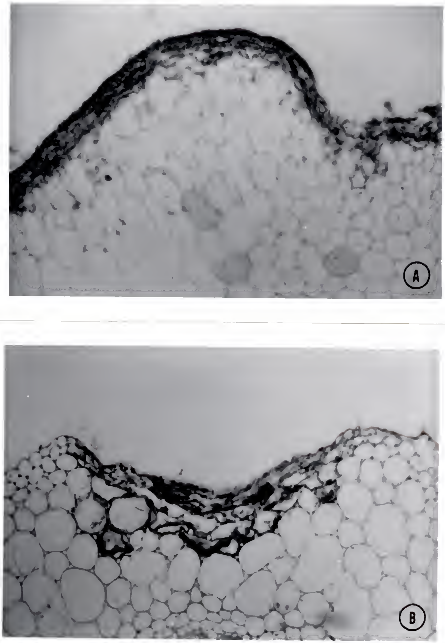

Fig. 15. Cross sections of immature tissues of eggplant showing their response to infection by Diaporthe vexans. A) Section of an infected stem of a seedling showing elongation of the cells below the infected area on the left and no cell elongation on a depressed area on the right (125X). B) Section of an infected petiole with a depressed lesion showing no sign of elongation, enlargement, or division of cells adjacent to infected cells (125X).

stems showed that epidermal and cortical cells invaded by the fungus were collapsed. The affected cells turned light to dark brown and this was manifested outside as a symptom. Adjacent to these collapsed dead cells were cells whose walls on the sides of the affected ones were showing early signs of discoloration. The subsequent layer of cells were mostly normal and showed no signs of division (Fig. 15-B). In some instances, there was a pronounced elongation of these cells, but there was no cell division.

Sections of raised lesions with brown or black centers showed divisions of normal cells adjacent to and away from infected cells (Fig. 16-A). Early infection involved only a few cells around the site of infection. Later, however, hyperplasia and hypertrophy occurred until a raised area became manifest on the affected part of the plant. Invaded epidermal cells often divided (Fig. 16-B). The next layer of cells, the chlorenchyma, also divided, but this tissue was almost always readily destroyed. Cells of the collenchyma, cortical parenchyma, as well as the phloem and xylem parenchyma also divided (Fig. 10, 16-A, 17). Prior to division, the thick-walled collenchyma either enlarged or became elongated and their walls became thin. Other parenchymatous cells divided with or without prior enlargement or elongation. Division occurred in various ways, but most of the elongated collenchyma divided periclinally.

As stated earlier in this paper, the fungus may be able to penetrate these barriers of newly divided cells and invade

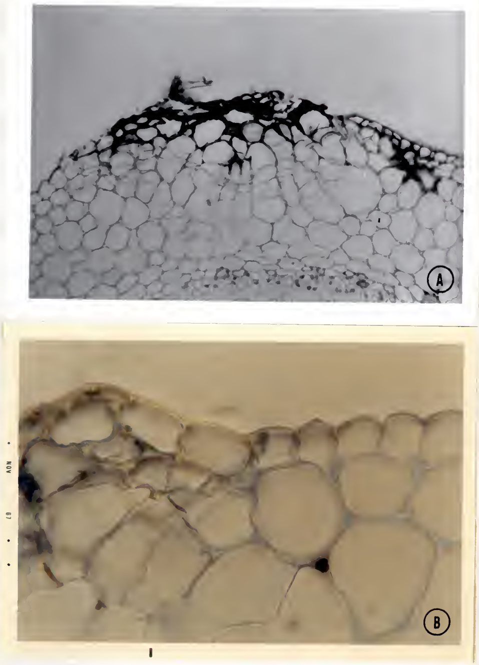

Fig. 16. Cross sections of mature tissues of eggplant showing their recation to infection by Diaporthe vexans. A) Section of a stem showing cell division adjacent to infected cells. Cell division has almost reached the phloem area (125X). B) Closer view of a stem section stained with lactophenol blue showing division of epidermal and collenchymatous cells (500X).

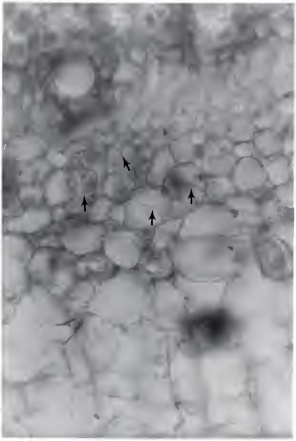

Fig. 17. Cross section of an eggplant stem infected by Diaporthe vexans in a more advanced state of infection showing (arrows) division of parenchyma of the phloem (500X).

the vascular elements. Death of stem or leaf follows. This occurred usually in immature plants. In mature plants, however, this meristematic region was able to halt the further advance of the fungus. A periderm-like tissue developed toward the outside. Thus, in some mature seedlings, the fungus was stopped at the cortex and some of the cortical cells remained unaffected (Fig. 18). The vascular elements continued to grow and develop secondary vessels. Although these plants escaped death, they were stunted in growth.

These results show the similarity of response of the immature petioles and stems with the immature seedlings to infection by the pathogen. In this case, however, more cell division and cell enlargement occurred several cells away from the infected region. Moreover, the fungus was unable to penetrate these cells.

Histology of Eggplant Tissues Injured Mechanically The histologic response of mature tissues to infection by the pathogen suggests that something other than simple mechanical injury induced other cells to divide and enlarge. In order to verify this, the histologic response of eggplant tissues injured mechanically was compared with tissues infected by the pathogen.

Mature petioles and stems were injured by pin-pricks and incubated in a plastic chamber. After a week, the mechanically injured areas were sectioned and examined. Cells that were ruptured by the pin pricks were killed and the cells adjacent to dead cells had enlarged and divided. Division was limited to only 1 or 2 layers of cells adjacent to injured cells (Fig. 19).

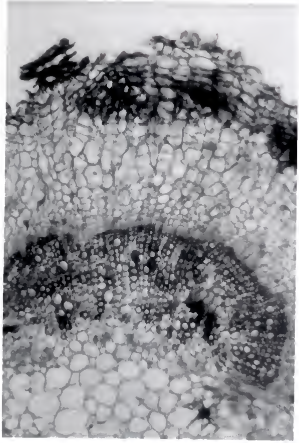

Fig. 18. Cross section of a seedling stem of eggplant girdled with lesions caused by Diaporthe vexans. Cell division was stopped at the cortex and some of the cells were not affected (125X).

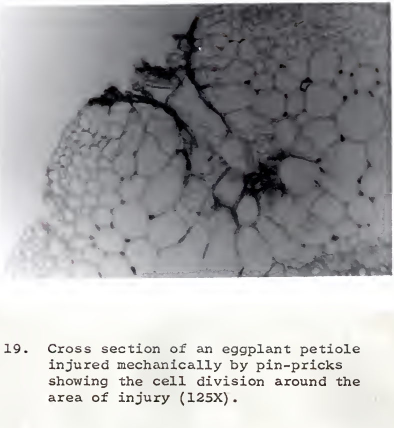

Fig. 19. Cross section of an eggplant petiole injured mechanically by pin-pricks showing the cell division around the area of injury (125X).

This result shows that the host responded to mechanical injury by cell enlargement and division only in the vicinity of the injured areas. This differs from the histology of tissues infected with the pathogen. Here, although cells adjacent to infected areas also enlarged, division induced many cells away from the infected areas. It shows, therefore, that a stimulus possibly coming from the fungus or caused to be elaborated in diseased cells may diffuse to other cells and induce these cells to enlarge and divide.

Pathological Histology of Solanum aff. Melongena and 'Fort Myers Market'

The results of the histological studies so far suggested that 'Florida Market' is no longer highly resistant as previously claimed. Further tests were made to compare the histological reaction of a variety less resistant than 'Florida Market' and another plant that is highly resistant to the pathogen. For a less resistant variety, 'Fort Myers Market' (Dr. P. Decker, personal communication) was used and for a highly resistant plant, *Solanum* aff. *melongena*, was chosen. One preliminary inoculation test showed that *S.* aff. *melongena* exhibited a high degree of resistance to the pathogen (Dr. A. A. Cook, personal communication).

Plants of 'Fort Myers Market' and *S.* aff. *melongena*, comparable in age to 'Florida Market,' when inoculated with the pathogen, exhibited symptoms similar to those shown by plants of 'Florida Market.' When sections of lesions from these plants were examined, cells adjacent to and away from infected ones also enlarged and divided. Fig. 20 shows the response of S. aff. melongena to infection by the pathogen. These observations indicate that 'Fort Myers Market' and S. aff. melongena have approximately the same degree of resistance as 'Florida Market' to the pathogen.

Histochemical Tests for Lignin and Suberin

The hypertrophy and hyperplasia of eggplant tissues, which resulted from the invasion by the pathogen and which eventually prevented the pathogen from further penetration, suggested that perhaps lignification or suberization of the newly divided cells occurred and provided a physical barrier to the pathogen. The following histochemical tests were conducted to test this hypothesis.

Stems and petioles showing various types of lesions were sectioned and stained for lignin by phloroglucinol and Schiff's reagent (24). Sections stained with phloroglucinol were observed within 30 min after placing the sections in the chemical. In the Schiff reaction, sections were examined 4 hr or longer after the start of the test. In sections of raised lesions, the most striking reaction took place at the boundary between the infected and the healthy cells. This area and the healthy mature xylem vessels showed a similar staining reaction (Fig. 21, 22). With phloroglucinol, this area was reddish-purple, and with Schiff's reagent, the color was darker red with a slight purplish tinge. On young lesions or during the early stage of the disease, all infected cells were stained either reddish-purple or red, depending upon the stain used. As infection

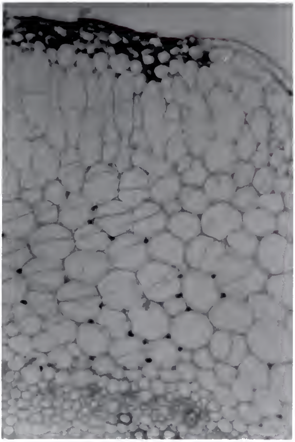

Fig. 20. Cross section of a stem of Solanum aff. melongena (horticultural variety) infected by Diaporthe vexans showing cell division of the cortex similar to that exhibited by S. melongena 'Florida Market' (125X).

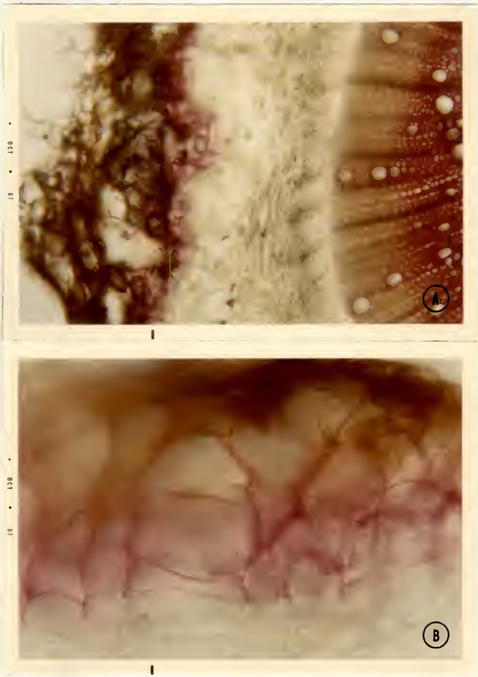

Fig. 21. Cross section of an eggplant stem infected by Diaporthe vexans and stained with phloroglucinol. A) Section showing the reddish-purple area between the diseased and healthy cells (125X). B) A closer view of the color reaction between the diseased and healthy areas (500X).

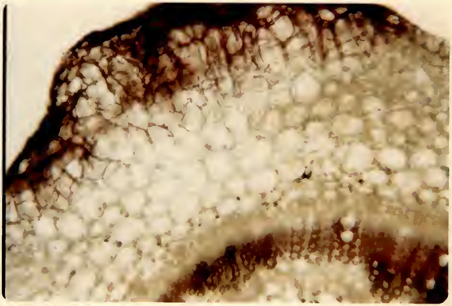

Fig. 22. Cross section of an eggplant stem infected by Diaporthe vexans and stained with Schiff's reagent showing the color reaction of the diseased and healthy areas (125X).

advanced inward, however, the cells on the outside turned brown, others black, and the next 1 or 2 layers became reddish-purple color to red (Fig. 21-B, 22). Healthy epidermal, cortical, and phloem tissues were colorless to greenish.

Sections of lesions from damped-off seedlings and sunken lesions of immature petioles and stems were also stained with phloroglucinol. Here, no reddish-purple color appeared on either infected cells or on the boundary between diseased and healthy cells (Fig. 23).

For suberin, Sudan dyes were used (24). The test was negative for suberin.

The phloroglucinol and Schiff tests on mature tissues were positive for lignin, indicating that the histogenic reaction physically checks the spread of fungus in affected tissues. Results are not conclusive, however. Although the phloroglucinol test is considered a standard test for lignin (24), there is a serious objection to it. Crocker (6) found that the phloroglucinol test does not indicate the presence of an appreciable part of the lignin molecule, but indicates the "traces of aldehyde which usually, perhaps always, accompany lignin." The Schiff reaction is also a test for the presence of aldehydes (24), and thus its significance falls in the same category as the phloroglucinol test. Until the chemistry of lignin is completely understood, the staining tests provide only an approximation of the presence of this compound.

The fact that no reddish-purple color appeared on lesions of immature susceptible tissues, suggests that whatever

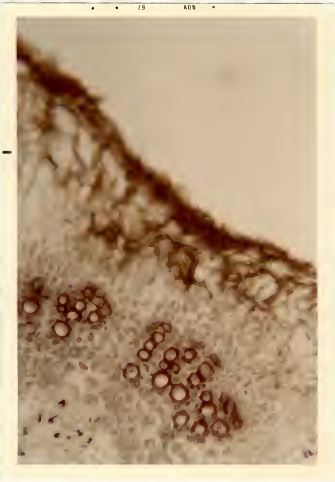

Fig. 23. Cross section of an immature eggplant petiole infected by Diaporthe vexans and stained with phloroglucinol showing no reddish-purple coloration between the diseased and healthy areas. The xylem vessels show a light reaction (125X).

produced the color is either not elaborated or not elaborated rapidly in immature tissues during pathogenesis.

Discussion

The fungus degraded pectic and cellulosic constituents of the host cell wall but since the degradation of these materials was slow, it is probable that these enzymes are produced only in low concentration. It is, however, equally probable that the fungus may produce a relatively high concentration of the enzymes but that they are partially inactivated by other substances. It is not known how important these enzymes are in killing cells of eggplant. If they are important, the partial inhibition of their activity may help to explain the failure of the fungus to penetrate farther in mature tissues that responded by cell enlargement and division to infection by the pathogen. As will be pointed out later, phenolic compounds may accumulate near infected cells; these compounds may retard the rates of pectolytic and cellulolytic activity.

The observations on the response of mature tissues to infection show that the fungus may stimulate cell division and enlargement by mechanical injury and may produce, or cause to be produced, some substances that easily diffuse to other cells and induce cell division and enlargement. The hyphae may cause cell enlargement and cell division by exerting mechanical pressure on cell walls while going from cell to cell. On the other hand, it is equally probable that chemical injury by the pectolytic and celluloytic enzymes, and perhaps other compounds, may also cause the same response. The host has reacted to infection by the pathogen in a manner which, according to Akai (1), is a dynamic defense reaction.

It is not conclusively shown whether the advance of the pathogen is stopped by the barricade of cells or by chemical means. There was no observable cork layer formation. The newly divided cells have thinner walls and hence would appear as susceptible as the original healthy cells. If, however, the stained area between the infected and healthy cells is truly lignified, this could provide a physical barrier for prevention of further penetration of the pathogen. It has been pointed out in other diseases that the metabolism of infected cells and the tissues nearby is increased and is followed by an accumulation of phenolic compounds (33, 38). The divisions occurring in the neighborhood of infected cells in this study attest to the increase in metabolic activity of this area. Thus, it is conceivable that phenolic compounds may accumulate in this area and may contribute to the restriction of the further advance of the pathogen. The phenols may either act as toxins to the pathogen or as inhibitors to enzymes produced by the pathogen and thus become a chemical barrier. Further, they could be used in the synthesis of lignin (33) and thereby contribute to the formation of a physical barrier.

Perhaps a re-evaluation of the Florida Market variety of eggplant as a supposedly resistant variety should be made. According to the present data, the variety is no longer highly resistant as previously claimed, but is only moderately resistant. This variety is shown to have no resistance to penetration by the pathogen, an observation consistent with a previous report that even resistant varieties were penetrated (23). Resistance is exhibited only after infection is initiated, but even then it is manifested only in mature tissues. Based on Dufrenoy's (11) categories of susceptibility or resistance, the present cytological evidences would class this variety as moderately susceptible. At the time of its release, 'Florida Market' was considered to be more resistant than standard varieties then in use (9, 10, 20). At that time, however, the hybrid was not pure, because it was still segregating for fruit color and type. It is probable that the variety has lost some degree of its resistance since its introduction in 1949.

A new breeding program to improve the level of resistance of 'Florida Market' should prove useful to the growers. Limited greenhouse inoculation experiments involving different species of Solanum* showed that S. pattersoni, S. miniatum, S. luteum, S. verbascifolium, and S. citrullifolium were not infected by the pathogen. No symptoms appeared on any of the inoculated plants. Other species like S. aculeatissimum, S. dulcamara, S. melanocerasum, S. sisymbrifolium, S. integrifolium, S. rostratum, S. heterodoxum, and S. pseudocapsicum were infected. Of these species infected, S. pseudocapsicum was

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*Seeds were obtained from a world-wide collection of the Virus Laboratory, Dept. of Plant Pathology, University of Florida. No attempt was made to verify the correct identification of the species.

was the least infected. In this species, only few superficial brown lesions were observed on veins and petioles of leaves.

Sources of resistance to the disease should come from species that showed no visible signs of infection and from the species that showed the least infection, provided, of course, that they can cross fertilize with eggplant. Other sources of resistance may also come from species that were infected. In this group, plants that will produce raised lesions may be used, since histological studies showed that, in eggplant, such lesions limit the internal spread of the pathogen. Furthermore, in screening work, plants showing sunken lesions may be discarded outright; no histologic defense mechanism is exhibited by such plants.

Further breeding work may now be aided by the knowledge that an abundance of inoculum can be produced easily in the laboratory. Growing the fungus in oatmeal, wheat, or rice agars from 20-30° C under constant illumination will assure the researcher an excellent supply of inoculum.

SUMMARY

D. vexans (Sacc. & Syd.) Gratz produced abundant pycnidia on oatmeal, rice, and wheat agars. On oatmeal agar, the pycnidia formed were smaller in size and solitary at low concentration and increased in size and tended to aggregate at higher oatmeal concentration. The fungus also needed light for abundant pycnidial production. It appears that the region close to the growing tip of a hypha is the most sensitive portion to the stimulatory effect of light. Overall growth and pycnidial production of this isolate was most satisfactory at 30° C. Mycelial growth at 35° C was inhibited, but pycnidial production was heaviest where mycelial growth has occurred.

Pycnidiospores of D. vexans germinated after 6 hr and entered the host after 12 hr. A penetrated cell may not become completely discolored until about 60 hr or more after inoculation at which time the first symptom of infection becomes evident. The fungus can infect all tissues, probably including the vascular elements. It spreads inside the suscept intra- and intercellularly. It is evidently a parasitic pathogen (facultative saprophyte), since it does not kill cells in advance of the hyphae.

The pathogen degraded pectic and cellulosic materials slowly suggesting that the enzymes responsible for degradation are either produced in low concentration or inactivated by other compounds. It is not known how important their role is in pathogenesis. Rapid death of cells occurred only after fungous penetration.

Immature seedlings are highly susceptible to infection. Mature tissues, however, show a dynamic type of resistance. The variety of eggplant, Florida Market, is not resistant to penetration. Although considered to be highly resistant, present cytological evidences suggest that the variety is only moderately resistant. There is need, therefore, for a renewed breeding of eggplant for resistance of Phomopsis disease.

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BIOGRAPHICAL SKETCH

Gil Gallego Divinagracia was born September 2, 1935, at Dumangas, Iloilo, Philippines. He graduated valedictorian from Dumangas High School in April, 1952. In March, 1956, he obtained the degree of Bachelor of Science in Agriculture, major in Plant Pathology from the University of the Philippines. He served as an Assistant Instructor in the Department of Agronomy at the same institution from June, 1956, to March, 1957, and as Graduate Research Fellow from June, 1957, to March, 1959, and Research Assistant from June, 1959, to September, 1960, in the Department of Plant Pathology. In September, 1960, he attended the University of California, Davis, under the sponsorship of the Rockefeller Foundation. He was conferred the degree of Master of Science in Plant Pathology from that institution in September, 1962. He returned to the Philippines and became Research Instructor in Plant Pathology at the University of the Philippines. Two years later, he was granted another scholarship by the Rockefeller Foundation. He entered the University of Florida in September, 1964, and since then has pursued his work toward the degree of Doctor of Philosophy to be granted in December, 1967.

He is a member of Phi Kappa Phi, Phi Sigma Biological Society, Gamma Sigma Delta, the Philippine Association for the Advancement of Science, and Alpha Zeta, and an associate member of the Sigma Xi, and the Society for the Advancement of Research (University of the Philippines). He is also a charter member and first secretary of the Philippine Phytopathological

Society, and a member of the American Phytopathological Society, and the Mycological Society of America.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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